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REPORT OF THE BIOLOGICAL LABORATORY

Eye and eye mucosa irritation study
in albino guinea pigs using
1-Methoxy-2-amino-4- β -hydroxyethylaminobenzene-sulphate





Study director:

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Darmstadt, August 21th 1979

Abstract

Test substance: 1-Methoxy-2-amino-4-β-hydroxyethylaminobenzene-sulphate, 1 percent aqueous solution

Object and method
of experiment:

It was the aim of the experiment to find out whether the diluted test substance would produce irritations of the eye and eye mucosa of albino guinea pigs upon single application to the eye.

Result:

Under the given experimental conditions, the test substance can be classified as "practically non irritating". Thus there are no objections to using the test substance in the eye region.

Beginning of
experiment:

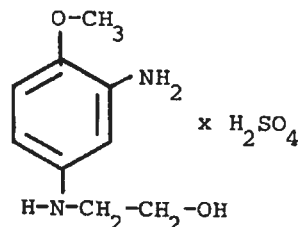
August 15th 1979

End of
experiment:

August 17th 1979

- I. Test substance 1-Methoxy-2-amino-4-β-hydroxyethylaminobenzene-sulphate, 1 percent aqueous solution

Supplier: Bayer, Leverkusen
Nature: fine, grey powder



II. Method

- A In analogy to the DRAIZE-TEST (1), 0.1 ml of the test substance was instilled into the conjunctival sac of the right eye. The left eye, remaining untreated, served as a control.

The test substance was not washed off. The following toxicity criteria were applied:

Injuries of the	I	conjunctiva
	II	cornea
	III	iris
	IV	fundus of the eye
	V	any general effect

The palpebral and bulbar mucosae were rated according to the seriousness of conjunctival swelling, redness, and discharge. The cornea was rated on the basis of the degree and area of opacity, and the iris was rated by the degree of inflammation. For the scale for scoring ocular lesions according to DRAIZE see page 8.

The fundus of the eye was examined for injuries of the retina and the papilla of the optic nerve.

Readings aids:

1. Magnifying glass
2. Ophthalmoscope (Heine Bifocal)
3. 1 %fluorescein-sodium solution (Merck, Darmstadt)

Ocular reactions were read and recorded 30 minutes, 1, 2, 3, 4, 6 and 7 hours after application. After 24 hours and then daily until the symptoms subsided, readings were made upon instillation of one drop of fluorescein-sodium solution diluted 1:100.

After each reading, the treated eyes were washed with a physiological sodium chloride solution.

B. Laboratory animals

10 female Pirbright white guinea pigs of the SPF-breed of Winkelmann*¹

Body weight: 380 - 400 g

Age: 7 - 8 weeks

The average life expectancy is 6 - 7 years, the average tumour rate is 0.5 to 1.5 %.

None of the animals had shown pathological alterations of the eyes before the experiment was started. The animals were randomized by means of an appropriate table (2).

Animal marking

All animals were ear-marked and the cages were provided with identification tags.

Substantiation of the choice of animals

Unlike the Draize-test, which suggests the use of rabbits, guinea pigs were used, because the Wella laboratory has been experimenting with the eyes of guinea pigs for many years. Another deviation from the Draize-test was the use of 10 animals instead of 3, in order to extend the safety margin of statistical evaluation and to avoid chance results.

C Housing

The animals were housed singly in a darkened room with uniform artificial lighting. The distance between the cages and the source of light was 1.30 to 2.50 m. For the intensity of light at the individual cages see the attached drawing, see page 6.

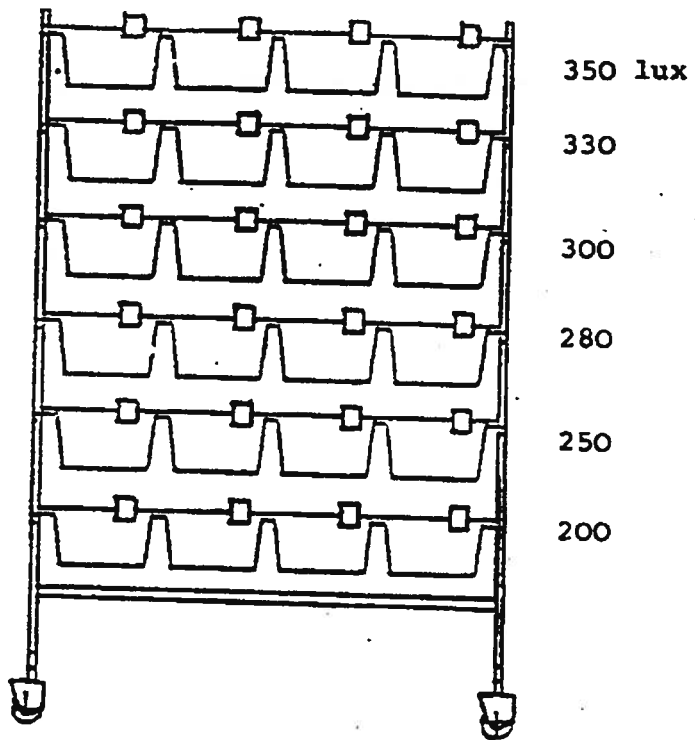
During the subsequent observation period the animals were kept in groups of 5 Macrolon cages, type IV (Ebeco*²) on standard "bedding" of "Ssniff"*³.

The animals were fed with a standard laboratory diet of Altromin*⁴; water was freely available at all times. The room temperature was $20^{\circ}\text{C} \pm 2^{\circ}$, the relative humidity $50\% \pm 5\%$ (maximum). Air circulation about 15 times per hour. Dark and light periods: 12 hours each.

Composition of laboratory diet: see page 18

Composition of water: see page 19

Intensity of light, determined with the luxmeter
Metrux 2 of METRAWATT AG, Nuremberg.



D Application

0.1 ml of the diluted test substance was instilled into the conjunctival sac of the right eye by means of a short pipette. The left eye remains untreated and served as a control.

24 hours after application, the eyes of all animals were washed with fluorescein-sodium solution in order not to miss any lesion invisible under normal circumstances.

E Determination of irritation index

The reactions of the eye and eye mucosa were read by two persons independently and the mean values were calculated. The reactions were scored according to the attached scale of DRAIZE (see page 8).

For determining the irritation index, the scores of the conjunctiva, cornea and iris were added at each reading and for each animal. The values of all animals were averaged. For classifying the test substance according to the verbal scores of table 3, the highest irritation index determined was taken into account. (3)



DATE

ARTICLE:

TEST-NO.

ANIMAL-NO.:

Guinea Pig right } eye
 left }

		hours after instillation								
		1/2	1	2	3	4	6	7	24	48
I. Conjunctivae										
A. Redness (refers to palpebral conjunctivae only)										
Vessels definitely injected above normal	1									
More diffuse, deeper crimson red, individual vessels not easily discernible	2									
Diffuse beefy red	3									
B. Chemosis										
Any swelling above normal (includes nictitating membrane)	1									
Obvious swelling with partial eversion of the lids	2									
Swelling with lids about half closed	3									
Swelling with lids about half closed to completely closed	4									
C. Discharge										
Any amount different from normal (does not include small amount observed in inner canthus of normal animals)	1									
Discharge with moistening of the lids and hairs just adjacent to the lids	2									
Discharge with moistening of the lids and considerable area around the eye	3									
Score (A + B + C) x 2 Total maximum = 20										
E. Cornea										
A. Opacity—Degree of Density (area which is most dense is taken for reading)										
Scattered or diffuse area—details of iris clearly visible	1									
Easily discernible translucent areas, details of iris slightly obscured	2									
Opalescent areas, no details of iris visible, size of pupil barely discernible	3									
Opaque, iris invisible	4									
B. Area of Cornea Involved										
One quarter (or less) but not zero	1									
Greater than one quarter—less than one-half	2									
Greater than one-half less than three quarters	3									
Greater than three quarters up to the whole area	4									
Score equals A x B x 5 Total maximum = 80										
III. Iris										
A. Values										
Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof) iris still reacting to light (sluggish reaction if positive)	1									
No reaction to light, hemorrhage; gross destruction (any or all of these)	2									
Score equals A x 5 Total possible maximum = 10										

The total score is the sum of all scores for

Conjunctiva max. 20
Cornea max. 80
Iris max. 10

maximum total score = 110

III. Results

1. General behaviour of the animals:

During the test and observation periods no abnormalities attributable to the treatment were observed. The food and water consumption as well as the development of the body weight were normal.

2. Reactions of the eye mucosa

- a) For individual findings in 10 female albino guinea pigs see table 1,
- b) For summary of ocular reactions see table 2.

Table 2

Irritation index (\bar{x}) at time of reading

Animal No.	Hours											
	1/2	1	2	3	4	6	7	24	48	72		
1	-	-	-	-	-	-	-	-				
2	-	2	2	-	-	-	-	-				
3	-	2	2	-	-	-	-	-				
4	-	-	2	-	-	-	-	-				
5	-	-	-	-	-	-	-	-				
6	2	-	-	-	-	-	-	-				
7	2	4	2	2	2	2	2	-				
8	2	4	2	2	2	2	2	-				
9	2	-	-	-	-	-	-	-				
10	2	-	-	-	-	-	-	-				
\bar{x} :	1,00	1,20	1,00	0,40	0,40	0,40	0,40	0				
s:	1,00	1,69	1,00	0,80	0,80	0,80	0,80	0				

Classification of the test substance according to the highest irritation index: $\bar{x} = 1,20$ see table 3

IV. Evaluation

Under the given experimental conditions, the test substance can be classified as "practically non-irritated".

No pathological alterations of the cornea, iris and fundus of the eye were observed.

The maximum irritation index was $\bar{x} = 1,2$ the total score of primary irritation being 110.

All reactions were faded away after 24 hours.



Table 3

Classification of the test substance according to the scores obtained in evaluating the irritation of the eye, with special regard to the scores after 24, 48, and 72 hours.

Score	Evaluation	For substantiating the classification, the following should be taken into account:
0.0-0.5	non-irritating	The 24-hour score should equal zero, otherwise include into next higher class.
0.5-2.5	practically non-irritating	The 24-hour score should equal zero, otherwise include into next higher class.
2.5-15	mildly irritating	The 72-hour score should equal zero, otherwise include into next higher class.
15-24	slightly irritating	On the 7th day of reading the score should equal zero.
25-50	moderately irritating	<p>a) On the 7th day of reading, the score of at least 60 % of the animals should be ≤ 10.</p> <p>b) The average on the 7th day should be ≤ 20.</p> <p>c) If the average $\bar{x} \leq 20$, but if less than 60 % of the animals have a score ≤ 10, none of the animals showing $\bar{x} > 10$, must score more than 30; otherwise include into the next higher class.</p>



Score	Evaluation	For substantiating the classification, the following should be taken into account:
50-80	strongly irritating	a) The 7th-day score should be 30 on at least 60 % of the animals. b) The 7th day score should be 40. c) If the average on the 7th day is 40, but if less than 60 % of the animals have a score >30, none of the animals showing $\bar{x} > 30$ must score more than 60; otherwise include into next higher class.
80-110	extremely irritating	

SUPPLIERS

- *1. Winkelmann
Versuchstierzucht GmbH & Co. KG
Gartenstraße 300

4791 Borcheln 1

- *2. Ebeco
E. Becker & Co. GmbH
Postfach 5 46

4620 Castrop-Rauxel

- *3. Ssniff-Versuchstier-Diäten GmbH
Thomätor 3

4770 Soest

- *4. Altromin GmbH
Tier-Labor-Service
Lange Straße 40

4937 Lage

REFERENCES

- (1) Draize, J.H., Dermal Toxicity, pp. 49-51,
Appraisal of the Safety Food and Drug Off.
of the United States, Topeka, Kansas, 1959.

- (2) Zufallszahlen - entnommen aus L. Sachs
Angewandte Statistik, Springer Verlag
Berlin, Heidelberg, New York 1973,
Seite 44, Tabelle 10

- (3) Kay, John H. and Calandra, Joseph C.,
"Interpretation of Eye Irritation Test",
Journal of the Society of Cosmetic Chemists,
Vo. XIII, No. 6, August 1962

altromin

Standard-diet
for Guinea-pigs

No.3020

Consistency:	flour	compact,	diameter (mm)		
		3	4.5	10	15
Order number:	3021	3022	-	-	3025
<u>Special diet:</u>	- Upon demand - Suitable for SPF, no pre-treatment required - In heat-sealed plastic bags.				
<u>Crude nutrients</u>		<u>Amino acids</u>			
Crude protein	18.0	Lysine	0.9		
Crude fat	4.3	Methionine + Cystine	0.5		
Crude fibre	13.5	Phenylalanin + Tyrosine	1.3		
Ash	8.9	Arginine	1.0		
Water	12.0	Histidine	0.4		
Nitrogen-free		Tryptophane	0.2		
extracts	43.3	Threonine	0.7		
Convertible energy:		Isoleucine	0.9		
Kcal/kg	2.700	Leucine	1.4		
KJ /kg	11.300	Valine	0.9		
<u>Minerals</u>		<u>Tracer elements</u>			
Calcium	1.0	Mangane	62.0		
Phosphorous	0.7	Iron	165.0		
Magnesium	0.2	Copper	16.0		
Sodium	0.2	Zinc	50.0		
		Jodine	0.9		
		Fluorine	10.0		
<u>Vitamins</u>		<u>Vitamins</u>			
<u>Standard-diet</u>		<u>Standard-diet (fortified) and</u>			
		<u>special diet</u>			
Vitamin A	15.000 IE	Vitamin A	25.000 IE		
Vitamin D ₃	600 IE	Vitamin D ₃	1.000 IE		
Vitamin E	75 mg	Vitamin E	125 mg		
Vitamin K ₃	3 mg	Vitamin K ₃	5 mg		
Vitamin B ₁	18 mg	Vitamin B ₁	30 mg		
Vitamin B ₂	12 mg	Vitamin B ₂	20 mg		
Vitamin B ₆	9 mg	Vitamin B ₆	15 mg		
Vitamin B ₁₂	24 mcg	Vitamin B ₁₂	40 mcg		
Nicotinic acid	36 mg	Nicotinic acid	60 mg		
Pantothenic acid	21 mg	Pantothenic acid	35 mg		
Folic acid	2 mg	Folic acid	3 mg		
Biotin	60 mcg	Biotin	100 mcg		
Choline	600 mg	Choline	1.000 mg		
Vitamin C	1.036 mg	Vitamin C	1.060 mg		

W a t e r

Chemical analysis

Type of water: Drinking water

Place of sampling: Darmstadt, Frankfurter Str. 100

Time of sampling: January-June 1979 (mean values)

Appearance: colourless clear Odour: inodourous

Total hardness: 16.1 °d.H. Carbonate hardness: 11.3 °d.H*

Consumption of potassium permanganate: 1.4 mg KMnO₄/litre

m-Value: 4.0

pH-Value: 7.3

1 litre of water contains ... mg of

Residue on evaporation (110°): --

Carbonic acid (CO₂):

Residue on ignition: --

Free CO₂: --

Calcium oxide (CaO): --

Combined CO₂: --

Calcium hardness: 12.2 °d.H

Magnesium oxide (MgO):

Aggressive CO₂: --

Magnesium hardness: 3.9 °d.H.

Aggressive CO₂ (Heyer): 0

Total iron content (Fe): < 0.05

Nitrates (NO₃'): 13.0

Iron, bivalent (Fe''): --

Nitrites (NO₂'): < 0.005

Iron, trivalent (Fe'''): --

Sulphates (SO₄'): 55

Manganese (Mn): < 0.03

Phosphates (P₂O₅): < 0.2

Aluminium (Al): --

Sulphides (S'): --

Ammonia (NH₃): < 0.05

Hydrogen sulfide (H₂S): --

Chlorides (Cl'): 25'

t₀ = 10.6°

Fluorides (F'): --

Silicic acid (SiO₂): 9.0

Oxygen (O₂): 6.5

SÜDHESSISCHE GAS UND WASSER AG, DARMSTADT
Laboratoriums-Leitung

* .1 degree of German hardness = 1.25 English hardness